

# Phenotypic characterization of rare interstitial deletion of chromosome 4

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**Abstract.** Interstitial deletion of the long arm of chromosome 4 is rare. Patients with interstitial deletion of the long arm of chromosome 4 differ from those with terminal deletions. Phenotypes may be variable, depending upon the specific length and location of the deleted portion. Here, we report on a boy exhibiting most of the congenital malformations encountered in terminal 4q syndrome. The conventional karyotyping and Fluorescence in-situ hybridization revealed a de novo interstitial del (4)(q31q32). The current report is a further document highlighting that deletion of segment q31 could be contributing to the expression of most of the phenotype of 4q deletion syndrome. Using array comparative genome hybridization methodology is recommended for investigating further cases with similar segmental interstitial deletions to support and delineate findings and to define genes implicated in the pathogenesis of the disorder.

**Keywords:** Chromosomal aberration, interstitial deletion chromosome 4(q31q32), phenotype, 4q terminal deletion syndrome

## 1. Introduction

Deletions of chromosomes are usually distinguished as interstitial or terminal. The severity of the phenotype correlates with the quantity of missing chromosomal material. The term 4q deletion syndrome has been used to describe patients who have deletion of the long arm of chromosome 4 detectable by standard karyotyping. Deletions of chromosome regions 4q31, 4q32, and 4q33-4qter lead to a distinctive malformation syndrome (deletion 4q syndrome) of facial dysmorphism, cardiac and limb defects and developmental delay [1]. More distal 4q deletions involving bands q34–q35 have been found in patients presenting with less

characteristic features and less severe mental retardation. Wagner et al. [2] suggested using the term 4q-syndrome for all cytogenetically visible deletions of the long arm of chromosome 4.

The deletion of the terminal region of the long arm of chromosome 4 was first described by Ockey et al. [3]. It was subsequently recognized as a distinct syndrome by Townes et al. [4]. The phenotype of this syndrome is well described in patients with a deletion of part of or all the terminal third of the long arm of chromosome 4 [5–7]. The lack of molecular characterization of the deletion sizes and deleted genes hinders further genotype-phenotype correlation [8]. To date there are over 100 patients with interstitial and terminal 4q deletion in the literature [8]. The chromosome deletions are mostly terminal. Interstitial deletions of 4q were less reported and most deletions had different breakpoints and variable clinical phenotypes.

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We report a new case with de novo small interstitial deletion of 4q (4q31-q32) in a male patient exhibiting most of the clinical manifestation encountered in terminal 4q syndrome.

## 2. Case report

A male patient at 6 mo, presented to our department because of congenital heart disease and clenched hands. He is the first and only offspring of first cousin consanguineous parents. His mother's and father's ages at time of his birth were 27 and 28 yr, respectively. Pregnancy history was uneventful. Delivery was by cesarean section because of prolonged and obstructed labor. Birth weight was 3 kg. He was incubated for 20 days because of respiratory distress. Family history is non-contributory.

On examination, the proband had dysmorphic features in the form of plagiocephaly, bilateral epicanthic folds, hypertelorism, upward slanting of palpebral fissures, depressed nasal root, broad nasal bridge small upturned nose, long and simple philtrum, retro-micrognathia and low-set ears (Fig. 1). General examination of chest, heart and abdomen were clinically normal, neurological examination showed normal muscle tone and preserved deep tendon reflexes. Patient has clenched hands, clinodactyly of all fingers and bilateral transverse palmar creases (Figs 2a and 2b). Feet showed arachnodactyly of toes and deep hallucal creases. At time of referral patient's weight was 5.5 kg ( $-2.7$  SD), length was 66 cm ( $-0.9$  SD), and head circumference



Fig. 1. Face of the patient showing bilateral epicanthic folds, hypertelorism, upward slanting of the palpebral fissures, depressed nasal root, broad nasal bridge, small upturned nose, long and simple philtrum, and low-set ears.



Figs. 2a and 2b. Hand of the patient is clenched and shows clinodactyly of all fingers and a transverse palmar crease.

was 39 cm ( $-2.9$  SD). Orodental examination showed pseudolabial cleft of lower lip, thick alveolar ridge, highly attached labial frenulum and a long bifid uvula.

Echocardiogram of the heart showed a small ventricular septal defect, atrial septal defect and patent ductus arteriosus, pulmonary hypertension, mildly dilated hypertrophied right ventricle and atrium. Abdominal and pelvic ultrasonography showed normal study. Patient had developmental delay. Serum calcium was 13 mg/dL (Normal: 8.1–10.4 mg/dL) and magnesium was 13 mg/dL (Normal: 1.8–2.6 mg/dL).

### 2.1. Cytogenetic studies

Conventional cytogenetic analysis following GTG banding technique was carried out for the proband on metaphases derived from phytohemagglutinin stimulated peripheral blood lymphocytes by standard methods described by Verma and Babu [9]. A total of 25 metaphases were karyotyped and analyzed according to ISCN [10]. The initial cytogenetic G-banding analysis revealed a reduction in length of the long arm of one chromosome 4 homologue, with an anomalous banding pattern at the distal end of the chromosome arm (Fig. 3). It was not clear whether it was a terminal or interstitial deletion by conventional cytogenetic methods. The karyotype was assumed as 46,XY,? del(4)(q31q32).

Fluorescence in-situ hybridization according to the method described by Pinkel et al [11] was performed on metaphase slides from the proband using the subtelomere 4q probe (Vysis). Hybridization and wash conditions followed the manufacturer's protocol, and at least five metaphase spreads were assessed for the

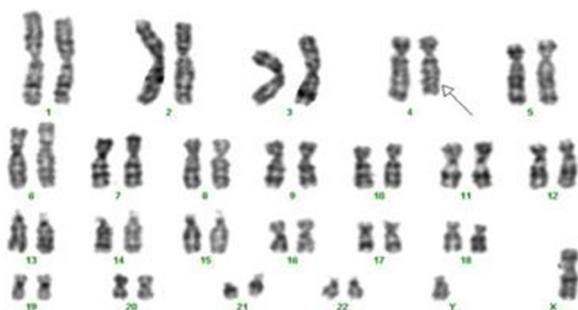


Fig. 3. G-banding karyotype of the proband showing 46,XY, del(4)(q31q32).

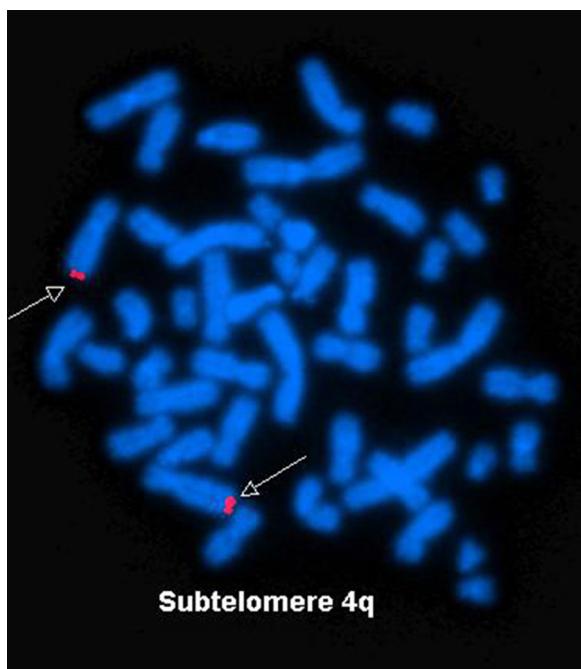


Fig. 4. Metaphase fluorescence in-situ hybridization using 4q subtelomeric probe (Vysis), showing two red signals in 4q.

presence of the subtelomeric region of chromosome 4q. A 4q sub-telomeric probe confirmed the interstitial nature of the deletion. Accordingly, the patient's karyotype is 46,XY,del(4)(q31q32), and parents karyotypes were normal (Fig. 4).

### 3. Discussion

Interstitial deletions of long arm of chromosome 4 are rare; most of the interstitial deletions involve the q11-q31 region. Few cases involving q31 or more

distal segments have been reported [12]. To our knowledge, the specific interstitial deletion of the segment q31-q32 has not been previously described. A review of literature showed few published reports of interstitial and terminal deletions involving a common segment with the current case.

Terminal 4q deletion (q31-pter), known as the 4q terminal deletion syndrome, has a relatively constant phenotype: craniofacial dysmorphisms including upward slanting of the palpebral fissures, hypertelorism, epicanthic folds, short upturned nose, long flat philtrum, thin lips, depressed nasal bridge, Pierre Robin sequence and abnormal external ears. Other major abnormalities include hand malformations (absent digits, clinodactyly, camptodactyly, abnormal thumb or hallux), abnormal palmar creases, congenital heart defects and genitourinary malformation [5,13,14].

Mental and growth retardation are consistently seen in patients with 4q31-pter deletion. In older children, learning disability and behavioral phenotype emerge with aggressive behavior, autistic and/or attention deficit and hyperactivity disorders [15].

The clinical manifestations of our patient were compared with that of 22 patients with terminal 4q deletion involving the breakpoint q31-pter [7,16], and with four cases having q32-pter [17] (Table 1). However, there was a clear difference regarding the frequency and severity of anomalies among the affected patients and our patient exhibited the major physical features encountered in 4q terminal deletion syndrome (Table 1). In addition, our patient had increased serum calcium, magnesium and hypercalcemia and such findings are rarely described in 4q deletion syndrome [18,19].

Review of the published reports showed few cases having interstitial deletion sharing a common deleted segment. Sarda et al. [12] reported a case with interstitial deletion (4q31-34) with severe cardiac, renal and skeletal defects, with less dysmorphic features compared to our case, with minimal digital malformation, absence of single palmar and deep hallucal creases. On the other hand, Sarda et al. [12] postulated that both the chromosome abnormality and maternal alcohol abuse contributed to the clinical findings of their case. They suggested that the characteristic phenotype attributed to terminal deletions of 4q31→pter probably results specifically from loss of q31→q33-34.

Aladhami et al. [20] reported a case with (del. 4q32-q33), with few common facial features and without visceral defects. Even the small hands and short fingers of their case could be attributed to the obesity of the patient (weight was > 97th centile).

Table 1  
4q terminal and interstitial deletion: A comparison of the common breakpoints of previous reports and the current case

Breakpoint	q31-qter	q32-qter	q31-q32*	q31-q34	q32-q33	q32-q34	q32-q34	q32-q34	Kaalund et al. [23]
Developmental delay	+	+	+	+	-	+	-	+	-
Growth retardation	+	+	+	+	-	-	-	-	-
Craniofacial	+	+	+	+	-	-	-	-	-
Abnormal skull	+	+	+	+	-	-	-	-	-
Hypertelorism									
Uplanting palpebral fissures									
Epicanticthic folds									
Broad nasal bridge									
Small upturned nose									
High arched palate									
Cleft lip/palate									
Micrognathia									
Ear abnormality									
Limbs									
Hand malformation									
Palmar crease									
Abnormal toes									
Visceral malformation									
Cardiac defect									
Genitourinary defect									
Major bone defect									

\* Our present case

However, their patient had initial normal developmental milestones, and then he developed learning and behavioral difficulties when he was 12 yr old. They pointed out that the more distal the deletion of the long arm of chromosome 4, the milder the phenotypes, the lower the incidence of major malformations and the rarer the early mortality as compared with less distal deletions. They also clarified the advantage of performing chromosome analysis in patients with learning difficulties and behavior problems who do not display major dysmorphic features.

Keeling et al. [21] reported a new case with de novo interstitial deletion 4q32-q34 who had Pierre Robin sequence, mild developmental delay, a left ulnar ray defect with absent ulna and associated metacarpals, carpals and phalanges and a right ulnar nerve hypoplasia. They proposed that genes for distal arm development, in particular for development of the left ulnar ray, central nervous system and cleft palate may be located at 4q33. Ramanathan et al. [22] described a child with an interstitial deletion of chromosome 4 (q32-q34), who had early developmental delay and minor dysmorphic features. His conventional karyotyping showed 46,XY,del (4q31.3-q33). The patient met the diagnostic criteria for autism when he was 11 yrs old. Molecular cytogenetic studies revealed a 19 megabase (Mb) deletion spanning 4q32-4q34. The 19 Mb deletion spans five sequenced contigs with 33 genes, among these 33 genes of known or inferred function, 13 genes are expressed in brain. They proposed that the deletion of the contiguous genes in the 4q32-4q34 region could lead to the specific dysmorphic features as well as potential candidate genes for autistic disorder. Such findings support the importance of karyotyping for patients with minimal dysmorphic features, learning and or behavioral problems.

Kaalund et al. [23] reported a patient with a de novo small interstitial deletion (4q32.1-q34.3) using array CGH confirmed by fluorescence in-situ hybridization. Their patient displayed the characteristic phenotype of the 4q deletion syndrome; they suggested that this deletion represents the 4q critical region. However, there is overlap with the deleted region of our patient and that of their reported patient, our patient has more dysmorphic features, more severe congenital heart malformations, hand malformation, and developmental delay and growth retardation characteristic of the 4q deletion syndrome.

Based on the genomic findings, an 11 Mb interval (170–181 Mb) of 4q33-q34.3 may harbor a candidate

gene for congenital heart disease [8]. Our case had atrial septal defect, ventricular septal defect, and patent ductus arteriosus suggesting that the candidate genes for congenital heart disease could be extended to a proximal deletion in the region 4(q31-q32). Huang et al. [24] suggested that cardiac phenotypes are very variable in patients with the terminal deletion of chromosome 4q.

Genomic characterization of additional cases with 4q distal deletions and other modifying genetic and epigenetic factors should help to clarify which genes and pathways are responsible for the spectrum of 4q deletion syndrome phenotypes [8]. Follow up of young patients like our patient is highly recommended to track the outcome regarding learning and or behavioral problems. Genomic characterization of patients with different breakpoints of distal 4q deletion could reveal potential candidate genes involved in facial, limb, cardiac, and central nervous system development, even learning and behavioral development. The current report is a further document highlighting that segment q31 could be responsible for the expression of most of the phenotype of 4q deletion syndrome.

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